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Lead tolerance in Collembola[1])

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With 2 figures

1. Introduction

A great number of studies have revealed significant accumulation of lead in soils adjacent to major roads. Uptake of lead appears to have variable effects in different species (*viz.* Ernst & Joosse 1983). Although human adults and mainly children are particularly susceptible to lead poisoning (Lorenzo *et al.* 1978; Landrigan & Baker 1981), with behavioural and intellectual disturbances (Budiansky 1981), only little effects of high lead levels in roadside soil have been found on the distribution and abundance of ground living fauna (Jefferies & French 1972; Williamson & Evans 1973; Maurer 1974; Rolfe & Jennett 1975; Getz *et al.* 1977; Wade *et al.* 1980). Even abnormally high doses of lead experimentally administered to Collembola did not result in death (Joosse & Buker 1979) and toxic effects in other evertebrate species have also still to be indicated (Williamson 1980).

In some organisms detoxification systems have been described. Worms have been shown to concentrate heavy metals in association with chloragocytes in the posterior alimentary tract (Ireland & Richards 1977). These cells have heavy metal binding properties (Ireland 1978), but the lead sequestration coincides with a decrease in glycogen reserves. Thus it could be hypothesised that the detoxification is an energy demanding process (Richards & Ireland 1978). A similar system is described for snails, that accumulate lead in the midgut gland (Coughtrey & Martin 1977). In Collembola a lead store is described in intestinal cells (spherites) (Humbert 1974, 1977), which are regularly removed during the frequently occurring moultings (Joosse & Buker 1979).

Although these structures function as safeguards against toxic actions of the metals, the detoxification mechanism might cost energy, with possible harmful consequences for growth and reproduction. In the present study the sublethal effects of lead-contaminated food on growth and reproduction of *Orchesella cincta* (L.) (Collembola) are described. To get more evidence about a possible disturbance of energy releasing processes the metabolic rate was determined by measuring the oxygen consumption.

An important food component of *Orchesella cincta* consists of *Pleurococcus* spec. (green-algae) (Joosse & Testerink 1979). Algae and lichens concentrate lead in natural conditions (Laaksovirta *et al.* 1976; Saeki *et al.* 1977), but also in the laboratory. When algae are soaked in lead nitrate solution impressive concentrations can be reached within a few minutes (Joosse & Buker 1979). It can be assumed however, that the animals, like Isopods (Joosse *et al.* 1981), have evolved metal tolerance by avoiding contaminated food or reducing its uptake. Since it is virtually unknown to what concentrations the animals are exposed in natural conditions and whether an avoidance mechanism the lead uptake reduces, both the sublethal effects of continuous administration and of a single dose of lead have been studied, and the behavioural response of the springtails to different lead concentrations was analysed.

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2. Materials and methods

2.1. General

A total of about 5,000 individuals of *Orchesella cincta* (L.) were collected in the Spanderswoud near Hilversum, the Netherlands, during different occasions. The animals were stored at 5 °C and, prior to the experiments, acclimated during 14 d to the experimental conditions of 20 °C or 15 °C (in exp. II) and 12L/12D. Four experiments were performed:

2.2. Uptake and excretion of lead

The animals were collected in August 1976. Four groups of about 950 individuals each, were treated in a different way: A was immediately killed, B, C and D ware fed for 2 d on lead contaminated food, B was subsequently killed, C was provided with clean food for another 2 d and C for another 4 d. Lead was extracted from the bodies and dissolved in seven parts nitric acid and one part perchloric acid and estimated with an AAS (Unicam SP 1900). A calibration curve was made using standard lead nitrate solutions. All concentrations are expressed in percentages of dry weight.

The food consisted of *Pleurococcus* spec. (green algae), which were soaked during 5 min in a lead nitrate solution of 1,000 ppm.

2.3. Effects of continuous lead administration on growth and respiration

The animals were collected in December 1979. Two groups of 100 individuals were kept with ten together in PVC jars (⌀ 5 cm) with a layer (2 cm) of moistened plaster of Paris. The groups were given clean and lead contaminated food respectively. The algae were soaked in 25 ppm lead nitrate. Growth was determined by weighing 10 animals together on a Mettler ME-30 microbalance (sensitivity 1 μg). The measurements of oxygen consumption (standard metabolism) were made on 2×5 individuals, which were kept separately, on clean and lead containing food, respectively. A Cartesian Diver micro-respirometer (LINDESTRØM-LANG 1943; HOLTER 1943) was used in a climate room at 15 °C. The instrument had 10 diver chambers. Stopped divers (ZEUTHEN 1964) were used with gas volumes in the range 80—94 μl. Readings were made on each individual at intervals of 20–30 min and continued for about 4 h, twice a week, during 28 d. Weight-specific oxygen consumption rates were calculated by weighing the individuals on a Mettler ME-30 microbalance.

2.4. Effects of a single dose of lead

The animals were collected in November 1978. The food (algae) was soaked in 25 ppm lead nitrate. Two subgroups were distinguished, one on clean algae, the other on lead containing algae each containing 100 individuals. Growth was measured as in experiment II. Reproduction was measured twice a week, during 4 weeks, by counting the number of eggs produced.

2.5. Behavioural responses to lead contaminated food

The animals were collected in October 1976. Preference experiments were performed with 4×20 individuals, which were offered a choice between portions of algae treated with 4 concentrations of lead, the algae being soaked in 25, 100, 250 and 1,000 ppm lead nitrate, respectively, and one control (clean algae). Two groups were starved prior to the experiment. The number of springtails present in the different food portions were counted every 5 min during 3 h.

For consumption experiments 2×50 individuals were used, one group of 50 being starved during 7 d. The animals were kept with ten together in PVC jars (⌀ 5 cm) and their faecal production of 24 h was taken as a measure of consumption activity.

3. Results

3.1. Lead uptake and excretion

A diet, consisting of fresh *Pleurococcus spec.* (greenalgae) was supplemented with 1,000 ppm lead. Four groups of about 950 individuals received a different treatment (Table 1).

Table 1. Experiment design and body lead concentration of *Orchesella cincta* after different treatments with lead containing algae

Group	nr of ind.	treatment	% moulted	body burden (ppm)
A	± 950	control	0	41.5
B	± 950	2 d. Pb	0	1,247.6
C	± 950	2 d. Pb 2 d. clean	12	247.5
D	± 950	2 d. Pb 4 d. clean	100	90.2

The individuals of group A were killed immediately after sampling and served as a control group. Groups B, C and D received 2 d lead contaminated algae; group B was killed without expelling the gut contents, whereas group C was fed for another 2 d on clean food. During the 4 d 12% of the animals of group C moulted. Group D got after 2 d another 4 d clean food. At that moment 100% of the animals had produced an exuvium.

Comparing the groups B and C, the data show that most of the ingested lead is lost by the faeces. But also during the moult a considerable portion is lost (D compared with C). Nevertheless, some of the lead turns up in the body (D compared with A).

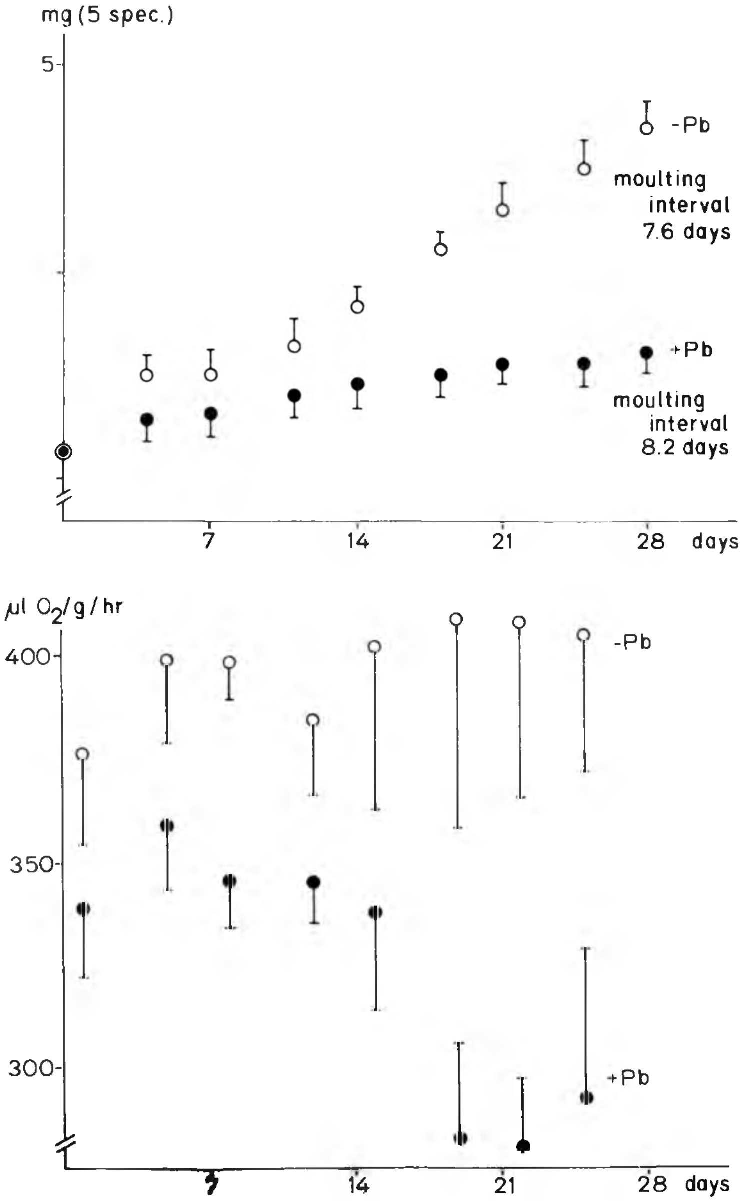

Fig. 1. Growth and weight specific oxygen consumption in *Orchesella cincta* on clean food and on food contaminated with 25 ppm lead nitrate.

3.2. Sublethal effects of continuous lead administration on growth and reproduction

In an experiment lasting four weeks the effect of lead contaminated food on growth and respiration was studied. The algae, soaked in a 25 ppm solution of lead nitrate concentrated the lead up to 8,400 ppm. Mortality in both groups was only slightly more than 1%. Results on growth and metabolic rate are presented in Fig. 1. Growth appeared to be significantly slower in the animals fed with lead containing food ($P < 0.001$) and the duration of the moulting interval, calculated on the basis of the number of exuviae produced, was found to be shorter. The oxygen consumption, observed on 5 separate individuals with the same treatment, also lowered as a result of lead exposure. Already after 2 d the metabolic rate (weight specific oxygen consumption) was less in the lead containing group, and subsequently decreased strongly during the experiment. The data show high variation, which is related to the physiology of moulting. Each individual measured, was in a different phase of the moulting interval and the oxygen consumption strongly fluctuates during the moulting interval (TESTERINK 1982a).

3.3. Sublethal effects of a single lead exposure on growth and reproduction

An experiment lasting four weeks was performed with animals fed for 4 d on algae, soaked in 25 ppm lead nitrate, which appeared to concentrate the lead up to 3,709 ppm. Afterwards the animals were fed with clean *Pleurococcus spec.* At the end of the experiment the body burden in both groups was 32 ppm ($n = 2$). Apparently after four weeks the lead was totally removed from the body. The effects of the temporary lead administration on growth and reproduction are summarized in Table 2.

Table 2. Effects of lead contaminated food on growth and reproduction in *Orchesella cincta*

Food	Increase in body weight/ ind./4 weeks (± s.e.)	duration moulting interval (days)	number of eggs/ 100 ind.
− Pb	4.37 ± 0.57 (n = 10)	5.0	3,099
+ Pb	4.57 ± 0.14 (n = 10)	4.9	2,266

Growth seems to be somewhat lower in the lead group, but the difference with the control group is not significant ($F_{[1,8]} = 0.06$). The difference between the duration of the moulting intervals is also insignificant. The reproduction seems to be negatively influenced by lead, although again the difference between both groups is not significant (Mann Whitney U-test).

3.4. Behavioural responses to lead contaminated food

3.4.1. *Preference experiments*

Twenty individuals of *O. cincta* were offered a choice between clean algae and algae soaked in 25, 100, 250 and 1,000 ppm lead nitrate, respectively. A comparison was made between the choices of normally fed animals and animals which had been starving for 7 d. Starvation is a normal and regular occurring phenomenon in natural populations of some species of Collembola, both related to the frequent moultings and as an adaptation to dry circumstances (JOOSSE & TESTERINK 1979; TESTERINK 1981). The results, presented in Fig. 2, show a capacity to discriminate between clean and lead containing food. A difference between the various concentrations is not clear. This can be explained by the property of algae to concentrate the lead to very high values, even when soaked in low concentrations of lead nitrate; apparently all contaminated food portions were equally unattractive. The difference between the fed and starved groups indicates that starved animals make a less dainty choice ($P < 0.005$, χ^2).

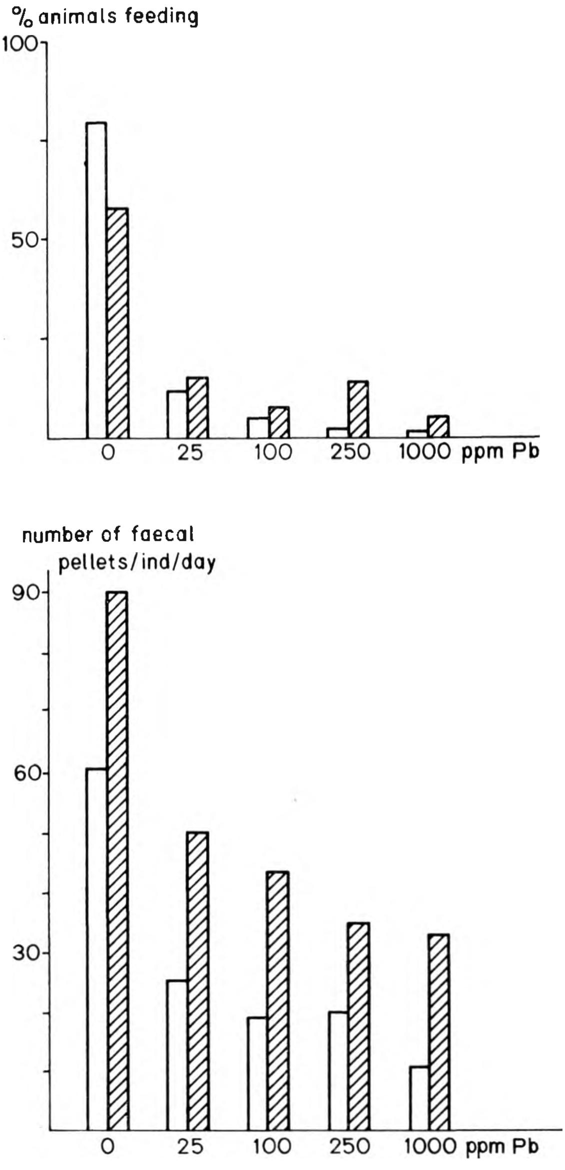

Fig. 2. Preference and consumption of *Orchesella cincta* on food, treated with different concentrations of lead nitrate. Open columms = normally fed animals; dashed columns = starved animals.

3.4.2. *Consumption*

Since a quantitative separation of food and faeces is virtually impossible, food uptake cannot be measured directly. The number of faecal pellets was taken as an indirect indication of the consumption activity. Fifty fed and 50 starved animals, kept individually, were fed during one day on the five concentrations mentioned above, 10 per concentration. Some of the individuals preparing a moult, did not produce faecal pellets (DE WITH & JOOSSE 1971). These data were omitted. The results are given in Fig. 2. The consumption of starved individuals appears to be significantly higher in all concentrations, but in both groups the consumption decreases with increasing lead concentration.

4. Discussion

A great number of studies suggest that ground living fauna along roads exhibit a high resistance to lead. Very little is known about the quantities of lead and the form in which it enters the bodies of saprophagous and herbivorous animals. In Collembola collected along a road where the lead content of the vegetation (grass) was 1,250 ppm, the body content was found to be 86 ppm (JOOSSE & BUKER 1979). For several other evertebrate species along roads values of a lower order are given (*viz.* ERNST & JOOSSE 1983). Some groups, and especially those with a high calcium need, like Isopods (MARTIN *et al.* 1976) and snails (MEINCKE & SCHALLER 1974; COUGHTREY & MARTIN 1976) seem to accumulate the lead up to higher values, indicating an interaction between calcium and lead. In worms it was shown that organelles, called chloragosomes, present in chloragogenous tissue around the intestine are capable of taking up and retaining lead and other heavy metals by a cation exchange system (IRELAND 1978). Afterwards the chloragocytes are broken down and excreted. A similar function may be found in particular intestinal cells in Collembola, where numerous intracellular mineral concretions can be found, which with the total renewal of the intestine during the moult, periodically are extruded (HUMBERT 1974; JOOSSE & BUKER 1979).

In the present study a value of 96 ppm comparable to that found in nature, was found in animals experimentally exposed to high levels of lead in their food. The data revealed that most of the lead was immediately removed with the faeces. This happens also in worms (IRELAND 1976) and woodlice (BEEBY 1978). Part of the lead however, turns up in the body. HUMBERT (1977) described that if the concentration of a toxic element is very high in the food of Collembola, it cannot be completely captured by the midgut concretions and enters the haemolymph, with possible harmful consequences. In the present experiments only a somewhat reduced reproduction was found after a single high dose of lead, of which a part must have passed the midgut barrier.

In worms excretions of heavy metals has been described as an energy demanding process (RICHARDS & IRELAND 1978). When the Collembola in this study were continuously exposed to high levels of lead in their food, growth was found to be reduced and a lowered metabolic rate indicated a disturbance of the energy releasing process. It is not known as yet, whether the lowered metabolic rate is caused by a direct inhibition of the activity of respiration enzymes. Since the food intake was also lowered with lead contaminated algae, it could also be related to the lower consumption (TESTERINK 1982b). These data do not allow a decisive answer about the energy costs of the tolerance mechanisms.

As one of the most numerous soil fauna groups of roadside verges, Collembola are most exposed to lead poisoning. Apparently these species have two forms of resistance mechanisms to lead, an avoidance and a tolerance mechanism (*viz.* LEVITT 1958; ERNST 1974). The avoidance mechanism implies the capability to discriminate between clean and contaminated food, and the regulation of the consumption, to avoid uptake of high quantities of lead. A tolerance mechanism is present in the intestinal cells, where lead is temporary bound and subsequently removed from the body.

5. Acknowledgements

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6. Zusammenfassung

Bleiverträglichkeit bei Collembolen

Zwei Arten von Resistenz-Mechanismen gegenüber Bleivergiftungen von Collembolen werden beschrieben. Erstens: Eine Fähigkeit zwischen reiner und kontaminierter Nahrung zu unterscheiden und eine Regulierung der Nahrungsaufnahme, die eine Aufnahme von hohen Bleidosen vermeidet. Zweitens: Es existiert ein Resistenz-Mechanismus in den Darmzellen, in denen Blei

vorübergehend gebunden und nach und nach aus dem Körper ausgeschieden wird. Zu hohe Bleidosen werden jedoch nicht vollständig gebunden und gelangen in die Hämolymphe. Eine andauernde Exposition gegenüber Blei beeinflußt das Wachstum und die Respiration der Collembolen negativ.

7. References

BEEBY, A., 1978. Interaction of lead and calcium uptake by the woodlouse *Porcellio scaber* (Isopoda, Porcellionidae). Oecologia (Berl.) **32**, 255—262.

BUDIANSKY, S., 1981. Lead: the debate goes on, but not over science. Environ. Sci. Technol. **15**, 243—246.

COUGHTREY, P. J., & M. H. MARTIN, 1976. The distribution of Pb, Zn, Cd and Cu within the pulmonate mollusc *Helix aspersa* MÜLLER. Oecologia **23**, 315—322.

— — 1977. The uptake of lead, zinc, cadmium and copper by the pulmonate mollusc, *Helix aspersa* MÜLLER, and its relevance to the monitoring of heavy metal contamination of the environment. Oecologia **27**, 65—74.

ERNST, W., 1974. Mechanismen der Schwermetallresistenz. Verh. Ges. Ökol., Erlangen, 189—197.

ERNST, W. H. O., & E. N. G. JOOSSE, 1983. Mineralstoffabhängige Belastung von Pflanze, Tier und Mensch. Jena.

GETZ, L. L., L. VERNER & M. PRATHER, 1977. Lead concentrations in small mammals living near highways. Environ. Pollut. **13**, 151—157.

HOLTER, H., 1943. Technique of the Cartesian Diver. C. R. Trav. Lab. Carlsberg Ser. Chim. **24**, 399—478.

HUMBERT, W., 1974. Localisation, structure et génèse des concrétions minérales dans le mésentéron des Collemboles Tomoceridae (Insecte, Collembole). Z. Morph. Tiere **78**, 93—109.

— 1977. The mineral concretions in the midgut of *Tomocerus minor* (Collembola): microprobe analysis and physioecological significance. Rev. Ecol. Biol. Sol **14**, 71—80.

IRELAND, M. P., 1976. Excretion of lead, zinc and calcium by the earthworm *Dendrobaena rubida* living in soil contaminated with zinc and lead. Soil Biol. Biochem. **8**, 347—350.

— 1978. Heavy metal binding properties of earthworm chloragosomes. Acta Biol. Acad. Sci. Hung. **29**, 385—394.

— & K. S. RICHARDS, 1977. The occurrence and localisation of heavy metals and glycogen in the earthworms *Lumbricus rubellus* and *Dendrobaena rubida* in a heavy metal site. Histochemistry **51**, 153—166.

JEFFERIES, D. J., & M. C. FRENCH, 1972. Lead concentrations in small mammals trapped on roadside verges and field sites. Environ. Pollut. **3**, 147—156.

JOOSSE, E. N. G., & J. B. BUKER, 1979. Uptake and excretion of lead by litter-dwelling Collembola. Environ. Pollut. **18**, 235—240.

— & G. J. TESTERINK, 1979. The role of food in the population dynamics of *Orchesella cincta* (LINNÉ) (Collembola). Oecologia **29**, 189—204.

— K. J. WULFRAAT & H. P. GLAS, 1981. Tolerance and acclimation to zinc of the isopod *Porcellio scaber* LATR. Proc. 3rd Int. Conf. Heavy Metals in the Environment, Amsterdam, 425—428.

LAAKSOVIRTA, K., K. OLKKONEN & P. ALAKUYALA, 1976. Observations on the lead content of lichen and bark adjacent to a highway in southern Finland. Environ. Pollut. **11**, 247—255.

LANDRIGAN, P. J., & E. L. BAKER, 1981. Exposure of children to heavy metals from smelters, epidemology and toxic consequences. Environ. Res. **25**, 204—224.

LEVITT, J., 1958. Frost, drought and heat resistance. In: HEILBRUNN, L. V., & F. WEBER (eds.), Protoplasmatologia, Handb. Protoplasmaforschung **8**, 6. Wien.

LINDESTRØM-LANG, K. U., 1943. On the theory of the Cartesian Diver microrespirometer. C. R. Trav. Lab. Carlsberg Ser. Chim. **24**, 333—398.

LORENZO, A. V., M. GEWIRTZ, D. AVERILL & M. MAURER, 1978. CNS lead toxicity in rabbit offspring. Environ. Res. **17**, 131—150.

MARTIN, M. H., P. J. COUGHTREY & E. W. YOUNG, 1976. Observations on the availability of lead, zinc, cadmium and copper in woodland litter and the uptake of lead, zinc and cadmium by the woodlouse *Oniscus asellus*. Chemosphere **5**, 313—318.

MAURER, R., 1974. Die Vielfalt der Käfer- und Spinnenfauna des Wiesenbodens im Einflußbereich von Verkehrsimmissionen. Oecologia **14**, 327—351.

MEINCKE, K. F., & K. H. SCHALLER, 1974. Über die Brauchbarkeit der Weinbergschnecke als Indikator für die Belastung der Umwelt durch die Elemente Eisen, Zink und Blei. Oecologia (Berl.) **15**, 393—398.

RICHARDS, K. S., & M. P. IRELAND, 1978. Glycogen-lead relationship in the earthworm *Dendrobaena rubida* from a heavy metal site. Histochemistry **56**, 55—64.

ROLFE, G. L., & J. C. JENNETT, 1975. Environmental lead distribution in relation to automobile and mine and smelter sources. In: KRENKEL, P. A., (ed.), Heavy metals in the aquatic environment. Proc. Int. Conf. Nashville, Tennessee, 231—241.

SAEKI, M., K. KUNII, T. SEKI *et al.*, 1977. Metal burden of urban lichens. Environ. Res. **13**, 256—266.

Testerink, G. J., 1981. Starvation in a field population of litter-inhabiting springtails (Collembola). Methods for determining food reserves in small arthropods. Pedobiologia **21**, 421—427.
— 1982a. Strategies in energy consumption and partitioning in Collembola. Ecol. Entom. **7**, 341—351.
— 1982b. Metabolic adaptations to seasonal changes in humidity and temperature in litter-inhabiting springtails. Oikos **39** (in press).
Wade, K. J., J. T. Flanagan, A. Currie & D. J. Curtis, 1980. Roadside gradients of lead and zinc concentrations in surface-dwelling invertebrates. Environ. Pollut (ser. B), **1**, 87—93.
Williamson, P., 1980. Variables affecting body burdens of lead, zinc and cadmium in a roadside population of the snail *Cepaea hortensis* Müller. Oecologia **44**, 213—220.
— & P. R. Evans, 1973. A preliminary study of the effects of high levels of inorganic lead on soil fauna. Pedobiologia **13**, 16—21.
With, N. D. de, & E. N. G. Joosse, 1971. The ecological effects of moulting in Collembola. Rev. Ecol. Biol. Sol **8**, 111—117.
Zeuthen, E., 1964. Microgasometric methods: Cartesian Divers. In: Schiebler, T. H., A. G. E. Pearse & H. H. Wolf, (eds.), 2nd Int. Congr. Histo- and Cytochemistry. New York, 70—80.

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Two forms of resistance mechanisms to lead poisoning in Collembola are described. First, a capability to discriminate between clean and contaminated food and a regulation of the consumption, avoids uptake of high doses of lead. Second, a tolerance mechanism exists in the intestinal cells, where lead is temporary bound and subsequently removed from the body. Too high doses of lead are not completely bound and enter the haemolymph. A continuous exposure to lead affects growth and respiration negatively.

Key words: Collembola, Insecta, Apterygota, lead, resistance, tolerance, poisoning, contamination, food.